Patent claims

1. A method for synthesising a bifunctional complex comprising an encoded molecule and an identifier polynucleotide identifying the chemical entities having participated in the synthesis of the encoded molecule, said method comprising the steps of

   i) providing

      a) at least one template comprising one or more codons capable of hybridising to an anti-codon, wherein said template is optionally associated with one or more chemical entities, and
      b) a plurality of building blocks each comprising an anti-codon associated with one or more chemical entities, and

   ii) hybridising the anti-codon of one or more of the provided building blocks to the template,

   iii) covalently linking said anti-codons and/or linking the at least one template with the anti-codon of at least one building block, thereby generating an identifier polynucleotide capable of identifying chemical entities having participated in the synthesis of the encoded molecule,

   iv) separating the template from one or more of the anti-codons hybridised thereto, thereby generating an at least partly single stranded identifier polynucleotide associated with a plurality of chemical entities,

   v) generating a bifunctional complex comprising an encoded molecule and an identifier polynucleotide identifying the chemical entities having participated in the synthesis of the encoded molecule,

      wherein said encoded molecule is generated by reacting at least two of said plurality of chemical entities associated with the identifier polynucleotide,

wherein said at least two chemical entities are provided by separate building blocks.

2. The method of claim 1, wherein the hybridisation of a first anti-codon to the template occurs sequentially or simultaneously with the hybridisation of a second anti-codon to the template.

3. The method of claim 1 or 2, wherein the hybridisation of a first anti-codon to the template occurs sequentially or simultaneously with the linkage of the first anti-codon to a second anti-codon or to the template.

4. The method of any of claims 1 to 3, wherein the hybridisation of a first anti-codon to the template occurs sequentially or simultaneously with the linkage of a second anti-codon to a further anti-codon or to the template.

5. The method of any of claims 1 to 4, wherein the linkage of a first anti-codon to the template occurs sequentially or simultaneously with the linkage of the first anti-codon to a second anti-codon.

6. The method of any of claims 1 to 5, wherein the linkage of a first anti-codon to a second anti-codon occurs sequentially or simultaneously with the linkage of the template to the second anti-codon.

7. The method of any of claims 1 to 6, wherein the template is separated from said covalently linked anti-codons by chemically or enzymatically cleaving one or more nucleotide linking bonds of the template.

8. The method of any of claims 1 to 6, wherein the template is non-covalently associated with the covalently linked anti-codons.

9. The method of claim 8, wherein the template is separated from said covalently linked anti-codons in a separation step selected from the group consisting of i) a step involving heating the template and the covalently linked anti-codons, thereby displacing the template from the covalently linked anti-codons, and ii) a step involving washing the template and the covalently linked anti-codons in a solvent

resulting in displacing the template from the covalently linked anti-codons, wherein said steps are optionally followed by one or more washing steps.

10. The method of any of claims 1 to 9, wherein at least one of said covalently linked anti-codons is further linked to a solid support, wherein the template is hybridised to the covalently linked anti-codons without being covalently linked to said covalently linked anti-codons, and wherein the template is separated from the covalently linked anti-codons by a step involving heating the template and the covalently linked anti-codons and/or a washing step resulting in physically separating the template from the covalently linked anti-codons.

11. The method of claim 10, wherein the template is linked to a member of an affinity pair.

12. The method of any of claims 1 to 9, wherein the template is linked to a solid support, wherein said covalently linked anti-codons are hybridised to the template without being covalently linked to said template, and wherein the covalently linked anti-codons are separated from the template by a step involving heating the template and the covalently linked anti-codons and/or a washing step resulting in physically separating the covalently linked anti-codons from the at least one template.

13. The method of claim 12, wherein at least one of said covalently linked anti-codons are further linked to one member of an affinity pair, wherein the other member of said affinity pair is linked to a further solid support, wherein the linkage of said affinity pair members results in attaching said covalently linked anti-codons to said further support.

14. The method of any of claims 7 to 13, wherein the identifier polynucleotide consists of covalently linked anti-codons.

15. The method of any of claims 7 to 13 wherein the identifier polynucleotide does not comprise the template, or a part thereof.

16. The method of any of claims 1 to 6, wherein the template is at least partly separated from said covalently linked anti-codons by chemically or enzymatically cleaving one or more nucleotide linking bonds of the template.

17. The method of claim 16, wherein the template is covalently associated with the covalently linked anti-codons.

18. The method of claim 17, wherein the template is at least partly separated from said covalently linked anti-codons in a separation step selected from the group consisting of i) a step involving heating the template and the covalently linked anti-codons, thereby displacing at least part of the template from the covalently linked anti-codons, and ii) a step involving washing the template and the covalently linked anti-codons in a solvent resulting in displacing at least part of the template from the covalently linked anti-codons, wherein said steps are optionally followed by chemically cleaving or enzymatically cleaving one or more nucleotide linking bonds of the template.

19. The method of any of claims 1 to 18, wherein the separation of at least part of said at least one template from covalently linked anti-codons hybridised to the template is carried out prior to the reaction of the at least two of said plurality of chemical entities.

20. The method of any of claims 1 to 6, wherein from 2 to preferably less than 100 building blocks are hybridised to at least one template, such as from 3 to preferably less than 50 building blocks are hybridised to at least one template, for example from 3 to preferably less than 20 building blocks are hybridised to at least one template, such as from 3 to preferably less than 10 building blocks are hybridised to at least one template, for example from 3 to preferably less than 8 building blocks are hybridised to at least one template, such as from 3 to preferably less than 7 building blocks are hybridised to at least one template.

21. The method of claim 20, wherein the reaction of chemical entities involve at least two reactive groups of at least some chemical entities.

22. The method of claim 1,

wherein the anti-codon of one of the provided building blocks is hybridised to the template,

wherein the anti-codon is covalently linked to the template,

wherein the anti-codon is displaced from the template, thereby generating an at least essentially single stranded identifier polynucleotide associated with a plurality of chemical entities,

wherein at least two of said plurality of chemical entities associated with the at least essentially single stranded identifier polynucleotide are reacted, thereby generating a bifunctional complex comprising a first encoded molecule and an identifier polynucleotide coding for chemical entities having participated in the synthesis of the first encoded molecule.

23. The method of claim 22 comprising the further steps of

i) hybridising the anti-codon of at least one further building block to the identifier polynucleotide of the first bifunctional complex generated in claim 7, wherein said anti-codon is associated with one or more chemical entities,

ii) covalently linking the anti-codon and the identifier polynucleotide of the first bifunctional complex,

iii) displacing the anti-codon from the identifier polynucleotide of the first bifunctional complex, thereby generating an at least essentially single stranded second identifier polynucleotide associated with the first encoded molecule and one or more chemical entities,

iv) reacting the first encoded molecule and the one or more chemical entities, and

v) generating a second bifunctional complex comprising a second encoded molecule and the second identifier oligonucleotide identifying the plurality of

chemical entities having participated in the synthesis of the second encoded molecule.

24. The method of claim 23, wherein steps i) to iv) are repeated for building blocks comprising different anti-codons and/or different chemical entities, thereby generating a plurality of bifunctional complexes comprising different encoded molecules.

25. The method of claim 24, wherein steps i) to iv) are repeated for building blocks comprising different chemical entities.

26. The method of any of claims 1 to 25, wherein the template comprises from 2 to preferably less than 100 codons, such as from 2 to preferably less than 10 codons.

27. The method of any of claims 1 to 25, wherein the template comprises from 3 to preferably less than 20 codons, such as from 3 to preferably less than 10 codons, for example from 3 to preferably less than 6 codons.

28. The method of any of claims 1 to 27, wherein each codon comprises or consists of a sequence of nucleotides.

29. The method of any of claims 1 to 28, wherein each codon comprises from 3 to 30 nucleotides.

30. The method of any of claims 1 to 29, wherein neighbouring codons are separated by a framing region.

31. The method of claim 30, wherein the framing region identifies the position of a codon.

32. The method of any of claims 30 and 31, wherein framing regions have alternating sequences.

33. The method of any of claims 1 to 32, wherein a template and/or at least one anti-codon further comprise one or more priming regions or regions capable of self-hybridisation.

34. The method of any of claims 1 to 33, wherein a template and/or at least one anti-codon further comprise one or more flanking regions, wherein said flanking regions optionally comprise a palindromic sequence of nucleotides capable of self-hybridisation, thereby forming a hair-pin loop structure.

35. The method of claim 34, wherein the template flanking region is at least partly complementary to the template priming region and allows the formation of a hair-pin loop structure comprising flanking region sequence hybridised to priming region sequence.

36. The method of any of claims 1 to 35, wherein the template comprises two PCR priming regions for amplification of the template.

37. The method of any of claims 1 to 36, wherein the plurality of building blocks each comprise an anti-codon covalently linked to at least one chemical entity.

38. The method of any of claims 1 to 37, wherein at least one of said building blocks comprise a chemical entity comprising a scaffold moiety comprising a plurality of reactive groups, and/or wherein the template is linked to a chemical entity comprising a scaffold moiety comprising a plurality of reactive groups.

39. The method of claim 38, wherein said scaffold moiety reactive groups react with one or more chemical entities of a single building block, or one or more chemical entities of different building blocks.

40. The method of claims 1 to 39, wherein the chemical entity of at least one building block is transferable to a recipient reactive group of a chemical entity of another building block, or a chemical entity linked to the template, preferably a chemical entity comprising a scaffold moiety comprising a plurality of reactive groups.

41. The method of any of claims 1 to 40, wherein at least one of said chemical entities can be selectively cleaved from the anti-codon of the building block.

42. The method of any of claims 1 to 41, wherein at least one chemical entity is simultaneously reacted with a reactive group of a recipient chemical entity and cleaved from the anti-codon to which the chemical entity is associated.

43. The method of any of claims 1 to 42, wherein at least one chemical entity forms one member of an affinity pair with another chemical entity.

44. The method of claim 43, wherein one of the affinity pairs is selected from biotin and dinitrophenol, and any derivative thereof capable of forming an affinity pair with a binding partner capable of forming said affinity pair with biotin and/or dinitrophenol.

45. The method of any of claims 1 to 44, wherein the anti-codon is protected at the 3' end and/or the 5' end by a protection group.

46. The method of any of claims 1 to 45, wherein at least one anti-codon is attached to a solid support, optionally via a 3' end protection group or a 5' end protection group.

47. The method of any of claims 1 to 46, wherein the template and/or the plurality of building blocks remain attached to a solid support during the synthesis of the bifunctional complex.

48. The method of any of claims 45 to 47, wherein the protection group is photo-cleavable.

49. The method of claim 48, wherein the protecting group is cleaved by exposure to UV light.

50. The method of any claims 45 to 49, wherein a phosphate group is formed at the 5' end of an anti-codon following deprotection thereof, thereby converting the anti-codon to a substrate for an enzyme comprising a ligase activity.

51. The method of any of claims 1 to 50, wherein one or more chemical entities are associated with the template.

52. The method of claim 51, wherein the one or more chemical entities are covalently linked to the template.

53. The method of any of claims 51 and 52, wherein the chemical entity linked to the template comprises a scaffold moiety.

54. The method of any of claims 1 to 7, wherein at least one anti-codon of a building block is further ligated to an oligonucleotide primer capable of complementing a priming region of the template.

55. The method of claim 54, wherein the oligonucleotide primer is further ligated to, or already covalently attached to, the template, thereby forming a covalent connection between the at least one anti-codon and the template.

56. The method of any of claims 1 to 55, wherein the at least one anti-codon comprises a sequence at least partly complementary to a framing sequence of the template.

57. The method of any of claims 1 to 56, wherein at least one building block or a subset of said plurality of building blocks are provided sequentially and/or sequentially hybridised to the template, wherein said sequentially provided and/or hybridised building block anti-codons are ligated, and wherein chemical entities of said subset of sequentially provided building blocks react before a further subset of building blocks are provided and/or hybridised to the template.

58. The method of any of claims 1 to 56, wherein all building block anti-codons are hybridised to the template simultaneously or in a single batch reaction.

59. The method of any of claims 1 to 58, wherein at least some building block anti-codons are ligated prior to or simultaneously with the reaction of chemical entities.

60. The method of any of claims 1 to 58, wherein at least some building block anti-codons are ligated before any of the chemical entities are reacted.

61. The method of any of claims 1 to 58, wherein all building block anti-codons are ligated before any of the chemical entities are reacted.

62. The method of any of the claims 1 to 61, wherein two or more building block anti-codons, such as 3 building block anti-codons, for example 4 building block anti-codons, such as 5 building block anti-codons, for example 6 building block anti-codons are hybridised to the template and subsequently ligated together to form an anti-codon ligation product.

63. The method of any of claims 1 to 62, wherein any subsequently hybridised building block anti-codon is hybridised in a neighbouring position to an already hybridised and optionally ligated anti-codon of a building block, or hybridised in a neighbouring position to an already hybridised and optionally ligated oligonucleotide primer, wherein said already hybridised building block anti-codon or oligonucleotide primer can be ligated to another building block anti-codon or to another oligonucleotide primer or to the template.

64. The method of any of claims 1 to 63, wherein at least some building block anti-codons are hybridised in a position spaced by one or more nucleotides from another building block anti-codon or oligonucleotide primer, and wherein a spacer oligonucleotide is provided and hybridised to the template for joining a building block anti-codon with a neighbouring building block anti-codon, or for joining a building block anti-codon with a neighbouring oligonucleotide primer.

65. The method of any of claims 1 to 64, wherein at least one of said plurality of building block anti-codons is immobilized on a solid support in the form of a beaded polymer.

66. The method of any of claims 1 to 65, wherein at least some neighbouring building block anti-codons are ligated by a chemical ligation reaction, thereby covalently linking said neighbouring building block anti-codons.

67. The method of claim 66, wherein the building block anti-codons linked by chemical ligation are selected from the group consisting of

a) first anticodons comprising a 3'-OH group and second anticodons comprising a 5´phosphor-2-methylimidazole group, which groups are reacted to form a phosphodiester internucleoside linkage,

b) first anticodons comprising a phosphoimidazolide group at the 3'-end and a phosphoimidazolide group at the 5'-end, which groups are reacted to form a phosphodisester internucleoside linkage,

c) first anticodons comprising a 3'-phosphorothioate group and second anticodons comprising a 5'-iodine group, which groups are reacted to form the internucleoside linkage 3'-O-P(=O)(OH)-S-5', and

d) first anticodons comprising a 3'-phosphorothioate group and second anticodons comprising a 5'-tosylate, which groups are reacted to form the internucleoside linkage 3'-O-P(=O)(OH)-S-5'.

68. The method of any of claims 1 to 65, wherein at least some building block anti-codons are ligated to the anti-codon of a neighbouring building block and/or to a template by a ligase, thereby covalently linking said building block anti-codons.

69. The method of claim 68, wherein the ligase is selected from the group consisting of DNA ligase and RNA ligase.

70. The method of claim 69, wherein the DNA ligase is selected from the group consisting of Taq DNA ligase, T4 DNA ligase, T7 DNA ligase, and *E. coli* DNA ligase.

71. The method of any of the preceding claims, wherein the at least essentially single stranded identifier polynucleotide is obtained by displacing codons and anti-codons under denaturing conditions resulting in said displacement.

72. The method of claim 71, wherein the denaturing conditions are obtained by performing the displacement in a media selected from organic solvents, aprotic solvents, acidic solvents, media comprising denaturants, and alkaline solvents.

73. The method of claim 72, wherein the denaturing conditions are obtained by heating the hybridised and covalently linked codons and anti-codons to a temperature

above the melting temperature of the duplex portion of the molecule, wherein said heating results in said displacement.

74. The method of claim 7 comprising the further step of degrading the template part of the identifier polynucleotide before any of the chemical entities are reacted.

75. The method of claim 74, wherein the template is an RNA template which is degraded by an enzyme selected from RNAseH, RNAseA and RNAse 1, by weak alkaline conditions (pH 9-10), or by aqueous $Pb(Ac)_2$.

76. The method of claim 74, wherein the template is a DNA template comprising an internucleoside linker comprising a thiophospate, wherein the template is treated with aqueous iodine.

77. The method of claim 74, wherein the template is a DNA template comprising an uracil nucleobase, wherein the template is treated with uracil-glycosylase and subsequently with weak acid.

78. The method of any of claims 1 to 77 comprising the further step of separating the template from a plurality of covalently linked anti-codons before reacting any chemical entities, reacting the chemical entities and generating a bifunctional complex comprising an encoded molecule and an identifier oligonucleotide consisting solely of ligated anti-codons, wherein said identifier oligonucleotide identifies the chemical entities having participated in the synthesis of the encoded molecule.

79. The method of claim 78, wherein the template is removed by cleaving at least one covalent link linking template codons and building block anti-codons, subjecting to cleavage product to conditions eliminating hybridisation between template codons and building block anti-codons, and separating the template from the covalently linked anti-codons.

80. The method of claim 79, wherein the covalent link is cleaved by a restriction endonuclease.

81. The method of any of claims 78 to 80, wherein the template comprises a first binding partner of an affinity pair, and wherein a second binding partner is optionally associated with a solid support, wherein said first and second binding partners constituted an affinity pair.

82. The method of any of claims 78 to 80, wherein at least one of said building blocks comprise a first binding partner of an affinity pair, and wherein the second binding partner is optionally associated with a solid support, wherein said first and second binding partners constituted an affinity pair.

83. The method of any of claims 81 and 82, wherein the binding of the binding partners of said affinity pair separates the template from the covalently linked anti-codons.

84. The method of any of claims 1 to 83 comprising the further step of separating codons and anti-codons by hybridising a nucleic acid to the template part of the molecule, thereby generating a duplex comprising the template.

85. The method of claim 84, wherein the duplex is provided by competition hybridisation by initially annealing a primer oligonucleotide to the template and extending said primer over the extent of the template using a polymerase.

86. The method of any of claims 1 to 85, wherein at least one chemical entity reaction is an acylation reaction.

87. The method of any of claims 1 to 85, wherein at least one chemical entity comprises an amine, and wherein an amide bond is formed when at least one chemical entitiy is reacted.

88. The method of any of the preceding claims comprising the further step of cleaving the encoded molecule from the identifier polunucleotide of a bifunctional complex.

89. The method of claim 1, wherein steps ii) through v) are repeated one or more times for building blocks comprising different anti-codons and/or different chemical entities, wherein said building block anti-codons hybridise to codons not already hybridised to an anti-codon in a previous synthesis round.

90. The method of any of claims 1 to 89, wherein the encoded molecule is associated with the template through a single bond.

91. The method of any of the preceding claims, wherein a plurality of bifunctional complexes are generated from the hybridisation of a plurality of templates to a plurality of building block anti-codons, covalently linking anti-codons hybridised to the same template, separating the template from at least some of the covalently linked anti-codons, preferably by degrading the template or by cleaving at least one chemical bond linking the template to the covalently ligated anti-codons followed by physical separation of the template and the covalently linked anti-codons, reacting the chemical entities and generating a library of bifunctional complexes each comprising a different encoded molecule and an identifier polynucleotide identifying the chemical entities having participated in the synthesis of the encoded molecule, wherein each of the plurality of encoded molecules are generated by reacting chemical entities associated with different anti-codons.

92. The method of claim 91, wherein pools each comprising a plurality of building blocks directed to each codon of the plurality of templates are added sequentially.

93. The method of claim 92, wherein different anti-codons in each pool have an identical flanking sequence.

94. A method for generating a library of different bifunctional complexes, said method comprising the steps of repeating the method of any of claims 1 to 90 using a different combination of building blocks and templates for each repetition.

95. The method of any of claims 91 to 94 comprising the further step of subjecting the library of bifunctional complexes to a partitioning procedure, such as an enrichment procedure and/or a selection procedure resulting in the enrichment and/or selection of bifunctional complexes displaying at least one desirable property.

96. The method of claim 95, wherein the enrichment procedure and/or selection procedure comprises the step of subjecting the library of bifunctional complexes to

a molecular target, and selecting bifunctional complexes binding to said molecular target.

97. The method of claim 95, wherein the enrichment procedure and/or selection procedure employs an assay generating for each bifunctional complex a result allowing a partitioning of the plurality of bifunctional complexes.

98. The method of any of claims 95 to 97 comprising the further step of obtaining the identifier polynucleotide part of a bifunctional complex from a plurality of said partitioned bifunctional complexes, optionally by separating the identifier polynucleotide from the encoded molecule of the bifunctional complex.

99. The method of any of claims 95 to 98 comprising the further step of amplifying in one or more steps said plurality of identifier polynucleotides by a linear amplification method or by an exponential amplification method, thereby generating a heterogeneous population of duplex molecules each comprising complementary identifier oligonucleotides identifying the chemical entities having participated in the synthesis of the encoded molecule of a bifunctional complex, wherein the identifier oligonucleotide is selected from the group consisting of identifier oligonucleotides comprising the template, or a part thereof, covalently linked to the covalently linked anti-codons, and identifier oligonucleotides comprising only covalently linked anti-codons and no template, or part thereof.

100. The method of any of claims 95 to 98 comprising the further step of converting said identifier polynucleotides into duplex molecules each comprising complementary identifier oligonucleotides identifying the chemical entities having participated in the synthesis of the encoded molecule of a bifunctional complex.

101. The method of any of claims 99 and 100 wherein the template part of the identifier oligonucleotide is separated from the encoded molecule prior to amplification.

102. The method of any of claims 99 to 101 comprising the further steps of displacing complementary identifier oligonucleotides, thereby generating a population of heterogeneous identifier oligonucleotides, and reannealing said

displaced identifier oligonucleotides under conditions where homo-duplexes and hetero-duplexes are formed, wherein homo-duplexes comprises identifier oligonucleotides originating from identical bifunctional complexes, and wherein hetero-duplexes comprises identifier oligonucleotides originating from different bifunctional complexes, such as bifunctional complexes comprising different encoded molecules.

103. The method of claim 102, wherein homo-duplexes and hetero-duplexes are separated by a chemical or enzymatical separation methods, or by physical separation methods.

104. The method of claim 103, wherein the homo-duplexes are isolated by removal of hetero-duplexes.

105. The method of claim 104, wherein the hetero-duplexes are removed by enzymatic degradation.

106. The method of claim 105, wherein the enzyme comprises a nuclease activity.

107. The method of any of the claims 105 and 106, wherein the enzyme is selected from T4 endonuclease VII, T4 endonuclease I, CEL I, nuclease S1, or variants thereof.

108. The method of any of claims 105 and 106, wherein the enzyme is thermostable.

109. The method of any of the claims 91 to 108, wherein the library comprises 1,000 or more different members, such as 10^5 different members, for example 10^6 different members, such as 10^7 different members, for example 10^8 different members, such as 10^9 different members, for example 10^{10} different members, such as 10^{12} different members.

110. The method of any of claims 96 to 109, wherein the molecular target is immobilized on a solid support.

111. The method of claim 110, wherein the target immobilized on the support forms a stable or quasi-stable dispersion.

112. The method of any of the claims 110 and 111, wherein the molecular target comprises a polypeptide.

113. The method of claim 112, wherein the polypeptide is selected from the group consisting of kinases, proteases, phosphatases.

114. The method of any of the claims 96 to 112, wherein the molecular target comprises an anti-body.

115. The method of any of the claims 96 to 110, wherein the molecular target comprises a nucleic acid.

116. The method of claim 115, wherein the nucleic acid comprises a DNA aptamer or an RNA aptamer.

117. The method of any of the claims 112 and 113, wherein the target polypeptide is attached to a nucleic acid having templated the synthesis of the polypeptide.

118. The method of any of claims 102 to 105, wherein any remaining homo-duplexes are amplified prior to decoding the identity of the encoded molecule of a bifunctional complex.

119. The method of claim 102, wherein the steps of identifier oligonucleotide displacement and reannealing are repeated at least once.

120. The method of any of claims 102 to 119, wherein identifier oligonucleotides comprising codons and/or anticodons are recovered from the selection procedure and reused for a second or further round synthesis of encoded molecules.

121. The method of claim 1,

wherein the anti-codons of from 3 to 8 building blocks are hybridised to a template sequentially or simultaneously in the same first compartment,

wherein at least one of the building blocks comprise a scaffold moiety comprising a plurality of reactive groups associated to an anti-codon,

wherein the template is covalently bound to a solid support, such as a beaded polymer,

wherein the covalently linked anti-codons are separated from the template covalently bound to the solid support, wherein said separation results in anti-codons and codons not being hybridised to each other,

optionally transferring the covalently ligated anti-codons to a second compartment, or transferring the template covalently bound to a solid support to a second compartment, and

reacting the chemical entities associated with the identifier polynucleotide, optionally in a compartment different from the compartment harbouring the template.

122. The method of claim 1,

wherein the anti-codons of from 3 to 8 building blocks are hybridised to a template sequentially or simultaneously in the same first compartment,

wherein at least one of the building blocks comprise a scaffold moiety comprising a plurality of reactive groups associated with an anti-codon,

wherein the covalently linked anti-codons are initially covalently linked to the template,

wherein the template part of the identifier oligonucleotide is degraded, thereby generating an identifier oligonucleotide comprising an essentially single stranded molecule comprising no template sequence,

optionally transferring the covalently ligated anti-codons to a second compartment, and

reacting the chemical entities associated with the identifier polynucleotide.

123. The method of claim 122, wherein the building blocks are provided sequentially, and wherein said method comprises the further steps of

i. covalently linking the anti-codon of a sequentially added building block to the template, or covalently linking the anti-codon of a sequentially added building block to an anti-codon covalently linked to the template,

ii. selecting a set of reaction conditions wherein codons and anti-codons do not hybridise to each other, thereby generating an essentially single stranded molecule,

iii. reacting a chemical entity of a sequentially added building block with a chemical entity associated with the template, or with a chemical entity associated with an anti-codon covalently linked to the template, and

iv. repeating steps i) to iii) for different building blocks.

124. A method for synthesising one or more bifunctional complexes each comprising a molecule resulting from the reaction of a plurality of chemical entities and an identifier polynucleotide identifying one or more of the chemical entities having participated in the synthesis of the molecule, said method comprising the steps of

i. providing a plurality of building blocks each comprising an oligonucleotide associated with one or more chemical entities,

ii. providing at least one connector oligonucleotide capable of hybridising with one or more building block oligonucleotides,

iii. immobilising at least one building block to a solid support,

iv. hybridising said immobilized building block oligonucleotide to a first connector oligonucleotide,

v. hybridising at least one additional building block oligonucleotide to said first connector oligonucleotide,

vi. ligating building block oligonucleotides hybridised to the connector oligonucleotide,

vii. separating the connector polynucleotide from the ligated building block oligonucleotides,

viii. reacting one or more chemical entities associated with different building block oligonucleotides, thereby obtaining a first bifunctional complex comprising a first molecule or first molecule precursor linked to a first identifier oligonucleotide identifying the chemical entities having participated in the synthesis of the molecule or molecule precursor, wherein said first bifunctional complex is immobilised to a solid support.

125. The method of claim 124, wherein said chemical entities are reacted in a reaction compartment from which the connector oligonucleotide has been removed in a washing and/or separation step prior to the reaction of said chemical entities.

126. The method of claim 124 comprising the further steps of

i. providing a second connector polynucleotide,

ii. hybridising said second connector polynucleotide to the identifier polynucleotide of said first bifunctional complex,

iii. hybridising at least one further oligonucleotide of a building block to said second connector oligonucleotide,

iv. ligating building block oligonucleotides hybridised to the second connector oligonucleotide, wherein at least one of said building block oligonucleotides are hybridised to the first identifier polynucleotide,

v. separating the second connector polynucleotide from the ligated building block oligonucleotides, for example by diverting the second connector polynucleotide to another compartment,

vi. reacting the first molecule precursor with the one or more chemical entities associated with the ligated building block oligonucleotide(s), thereby obtaining a second bifunctional complex comprising a molecule or molecule precursor linked to a second identifier polynucleotide identifying the chemical entities having participated in the synthesis of the molecule or molecule precursor, wherein said second bifunctional complex is immobilised to a solid support.

127. The method of claim 126, wherein steps i) to vi) are repeated for different connector oligonucleotides and different further building blocks.

128. The method of any of claims 124 to 127, wherein said bifunctional complex or a plurality of such complexes are released from the solid support.

129. The method of any of claims 124 to 128, wherein different bifunctional complexes are generated in different reaction compartment, and wherein at least some of said different bifunctional complexes are combined in a reaction compartment comprising a plurality of further connector oligonucleotides, wherein at least two of said different bifunctional complexes hybridise to a further connector polynucleotide, wherein the molecule precursor part of said complexes react, thereby generating a further molecule in the form of a reaction product, wherein the identifier polynucleotides of said bifunctional complexes are optionally covalently linked prior to or after the reaction of the molecule precursors, wherein the covalently linked identifier polynucleotides are optionally separated from the further connector oligonucleotide prior to or after reaction of said molecule precursors.

130. A method for synthesising a bifunctional complex comprising a molecule resulting from the reaction of a plurality of chemical entities, wherein said molecule is linked to an identifier polynucleotide identifying one or more of the chemical entities having participated in the synthesis of the molecule, said method comprising the steps of

i) providing a plurality of building blocks selected from the group consisting of

a) building blocks comprising an identifier oligonucleotide linked to one or more chemical entities,
b) building blocks comprising an identifier oligonucleotide linked to one or more reactive groups, and
c) building blocks comprising an identifier oligonucleotide comprising a spacer region, wherein said building blocks comprising a spacer region are preferably connector polynucleotides to which building blocks of groups a) and b) can hybridise,

ii) generating a hybridisation complex comprising at least n building blocks by hybridising the identifier oligonucleotide of one building block to the identifier oligonucleotide of at least one other building block,

wherein n is an integer of 4 or more

wherein at least 3 of said at least n building blocks comprise a chemical entity,

wherein no single identifier oligonucleotide is hybridised to all of the remaining identifier oligonucleotides,

wherein optionally at least one of said building blocks of group c) is immobilised to a solid support, thereby providing a handle to which an oligonucleotide of at least one building block of groups a) or b) can hybridise,

iii) covalently linking identifier oligonucleotides of building blocks comprising one or more chemical entities, thereby obtaining an identifier polynucleotide comprising covalently linked identifier oligonucleotides each associated with one or more chemical entities,

iv) optionally separating said identifier polynucleotide obtained in step iv) from any immobilised connector oligonucleotides hybridied thereto, wherein said separation optionally comprises the step of diverting said identifier polynucleotide comprising covalently linked identifier oligonucleotides each associated with one or more chemical entities to a different reaction compartment, thereby separating said identifier polynucleotide from said immobilised connector oligonucleotides

v) reacting said at least 3 chemical entities linked to the identifier polynucleotide, and

vi) obtaining a bifunctional complex comprising a molecule resulting from the reaction of a plurality of chemical entities, wherein said molecule is linked to an identifier polynucleotide identifying one or more of the chemical entities having participated in the synthesis of the molecule.

131. The method of claim 130 wherein a plurality of different bifunctional complexes is obtained by repeating the method steps for different building blocks.

132. The method of any of claims 124 to 131, comprising reacting at least 3 chemical entities, such as at least 4 chemical entities, for example at least 5 chemical entities, such as at least 6 chemical entities.

133. The method of any of claims 1 to 132,

wherein a plurality of molecules are synthesised,

wherein the plurality of synthesised molecules are selected from the group consisting of α-peptides, β-peptides, γ-peptides, ω-peptides, mono-, di- and tri-substituted

α-peptides, β-peptides, γ-peptides, ω-peptides, peptides wherein the amino acid residues are in the L-form or in the D-form, vinylogous polypeptides, glycopolypeptides, polyamides, vinylogous sulfonamide peptides, polysulfonamides, conjugated peptides comprising e.g. prosthetic groups, polyesters, polysaccharides, polycarbamates, polycarbonates, polyureas, polypeptidylphosphonates, polyurethanes, azatides, oligo N-substituted glycines, polyethers, ethoxyformacetal oligomers, poly-thioethers, polyethylene glycols (PEG), polyethylenes, polydisulfides, polyarylene sulfides, polynucleotides, PNAs, LNAs, morpholinos, oligo pyrrolinones, polyoximes, polyimines, polyethyleneimines, polyimides, polyacetals, polyacetates, polystyrenes, polyvinyl, lipids, phospholipids, glycolipids, polycyclic compounds comprising e.g. aliphatic or aromatic cycles, including polyheterocyclic compounds, proteoglycans, and polysiloxanes, including any combination thereof,

wherein each molecule is synthesised by reacting a plurality of chemical entitiespreferably in the range of from 2 to 200, for example from 2 to 100, such as from 2 to 80, for example from 2 to 60, such as from 2 to 40, for example from 2 to 30, such as from 2 to 20, for example from 2 to 15, such as from 2 to 10, such as from 2 to 8, for example from 2 to 6, such as from 2 to 4, for example 2, such as from 3 to 100, for example from 3 to 80, such as from 3 to 60, such as from 3 to 40, for example from 3 to 30, such as from 3 to 20, such as from 3 to 15, for example from 3 to 15, such as from 3 to 10, such as from 3 to 8, for example from 3 to 6, such as from 3 to 4, for example 3, such as from 4 to 100, for example from 4 to 80, such as from 4 to 60, such as from 4 to 40, for example from 4 to 30, such as from 4 to 20, such as from 4 to 15, for example from 4 to 10, such as from 4 to 8, such as from 4 to 6, for example 4, for example from 5 to 100, such as from 5 to 80, for example from 5 to 60, such as from 5 to 40, for example from 5 to 30, such as from 5 to 20, for example from 5 to 15, such as from 5 to 10, such as from 5 to 8, for example from 5 to 6, for example 5, such as from 6 to 100, for example from 6 to 80, such as from 6 to 60, such as from 6 to 40, for example from 6 to 30, such as from 6 to 20, such as from 6 to 15, for example from 6 to 10, such as from 6 to 8, such as 6, for example from 7 to 100, such as from 7 to 80, for example from 7 to 60, such as from 7 to 40, for example from 7 to 30, such as from 7 to 20, for example from 7 to 15, such as from 7 to 10, such as from 7 to 8, for example 7, for example from 8 to 100, such as from 8 to 80, for example from 8 to 60, such as from 8 to 40, for example from 8 to 30, such as from 8 to 20, for example from 8 to 15, such as from 8

to 10, such as 8, for example 9, for example from 10 to 100, such as from 10 to 80, for example from 10 to 60, such as from 10 to 40, for example from 10 to 30, such as from 10 to 20, for example from 10 to 15, such as from 10 to 12, such as 10, for example from 12 to 100, such as from 12 to 80, for example from 12 to 60, such as from 12 to 40, for example from 12 to 30, such as from 12 to 20, for example from 12 to 15, such as from 14 to 100, such as from 14 to 80, for example from 14 to 60, such as from 14 to 40, for example from 14 to 30, such as from 14 to 20, for example from 14 to 16, such as from 16 to 100, such as from 16 to 80, for example from 16 to 60, such as from 16 to 40, for example from 16 to 30, such as from 16 to 20, such as from 18 to 100, such as from 18 to 80, for example from 18 to 60, such as from 18 to 40, for example from 18 to 30, such as from 18 to 20, for example from 20 to 100, such as from 20 to 80, for example from 20 to 60, such as from 20 to 40, for example from 20 to 30, such as from 20 to 25, for example from 22 to 100, such as from 22 to 80, for example from 22 to 60, such as from 22 to 40, for example from 22 to 30, such as from 22 to 25, for example from 25 to 100, such as from 25 to 80, for example from 25 to 60, such as from 25 to 40, for example from 25 to 30, such as from 30 to 100, for example from 30 to 80, such as from 30 to 60, for example from 30 to 40, such as from 30 to 35, for example from 35 to 100, such as from 35 to 80, for example from 35 to 60, such as from 35 to 40, for example from 40 to 100, such as from 40 to 80, for example from 40 to 60, such as from 40 to 50, for example from 40 to 45, such as from 45 to 100, for example from 45 to 80, such as from 45 to 60, for example from 45 to 50, such as from 50 to 100, for example from 50 to 80, such as from 50 to 60, for example from 50 to 55, such as from 60 to 100, for example from 60 to 80, such as from 60 to 70, for example from 70 to 100, such as from 70 to 90, for example from 70 to 80, such as from 80 to 100, for example from 80 to 90, such as from 90 to 100.

134. The method of any of claims 1 to 132, wherein the molecule is a small molecule comprising a plurality of functional groups generating by reaction of a plurality of chemical entities, wherein said functional groups are be linked by one or more chemical bonds selected from the group consisting of chemical bonds such as peptide bonds, sulfonamide bonds, ester bonds, saccharide bonds, carbamate bonds, carbonate bonds, urea bonds, phosphonate bonds,urethane bonds, azatide bonds, peptoid bonds, ether bonds, ethoxy bonds, thioether bonds, single carbon bonds, double carbon bonds, triple carbon bonds, disulfide bonds, sulfide bonds,

phosphodiester bonds, oxime bonds, imine bonds, imide bonds, including any combination thereof.

135. The method of any of claims 1 to 132, wherein the molecule is a small molecule comprising a plurality of functional groups generating by reaction of a plurality of chemical entities, wherein said functional groups are be linked by one or more chemical bonds selected from the group consisting of -NHN(R)CO- ; -NHB(R)CO- ; -NHC(RR')CO- ; -NHC(=CHR)CO- ; -NHC_6H_4CO-; -$NHCH_2$ CHRCO-; -$NHCHRCH_2$ CO- ; -$COCH_2$- ; -COS- ; -CONR- ; -COO- ; -CSNH- ; -CH_2 NH- ; -CH_2CH_2- ; -CH_2 S- ; -CH_2 SO- ; -CH_2SO_2- ; -$CH(CH_3)$S- ; -CH=CH- ; -NHCO- ; -NHCONH- ; -CONHO- ; -C(=CH_2)CH_2- ; -PO_2^-NH- ; -$PO_2^-CH_2$- ; -$PO_2^-CH_2N^+$- ; -SO_2NH^-- ; and lactams, including any combination thereof.

136. The method of any of claims 124 to 135, wherein said method results in the synthesis of more than or about 10^3 different molecules, such as more than or about 10^4 different molecules, for example more than or about 10^5 different molecules, such as more than or about 10^6 different molecules, for example more than or about 10^7 different molecules, such as more than or about 10^8 different molecules, for example more than or about 10^9 different molecules, such as more than or about 10^{10} different molecules, for example more than or about 10^{11} different molecules, such as more than or about 10^{12} different molecules, for example more than or about 10^{13} different molecules, such as more than or about 10^{14} different molecules, for example more than or about 10^{15} different molecules, such as more than or about 10^{16} different molecules, for example more than or about 10^{17} different molecules, such as more than or about 10^{18} different molecules.

137. A method for synthesising a bifunctional complex comprising an encoded molecule and a template coding for one or more chemical entities which have participated in the synthesis of the encoded molecule, the method comprising the steps of

i) providing

a) a template comprising one or more codons

b) one or more building blocks having an anticodon associated with a chemical entity, and

c) a nucleic acid sequence associated with a reactive site,

ii) contacting the tempate with the one or more building blocks under conditions allowing for hybridisation between codons and anticodons,

iii) ligating at least one anticodon of a building block to the nucleic acid sequence associated with the reactive site, and

iv) reacting the chemical entity of the ligated building block with the reactive site under conditions where the ligation product is single stranded, to obtain a template-encoded reaction product.

138. The method according to claim 137, wherein the template comprises 2-100 codons.

139. The method according to claim 137 or 138, wherein the template comprises 3-20 codons.

140. The method according to claims 137 to 140, wherein the codon is a sequence of nucleotides.

141. The method according to any of claims 137 to 140, wherein each codon comprises 3-30 nucleotides.

142. The method according to claims 137 to 141, wherein neighbouring codons are separated by a framing region.

143. The method according to claim 141, wherein the framing region identifies the position of a codon.

144. The method according to claim 142 or 143, wherein the framing regions have alternating sequences.

145. The method according to any of the claims 137 to 144, wherein the template further comprises a priming region.

146. The method according to any of the claims 137 to 145, wherein the template further comprises a flanking region.

147. The method according to claims 146 or 147, wherein the flanking region is complementary to the priming region allowing for a hairpin loop to be formed.

148. The method according to any of the preceding claims, wherein two PCR priming regions are present on each side of the coding sequences.

149. The method according to any of the claims 137 to148, wherein the building block comprises an anticodon covalently connected to a chemical entity.

150. The method according to claim 137 to 149, wherein the chemical entity is a scaffold.

151. The method according to claims 137 to 150, wherein the chemical entity is transferable to a recipient reactive group.

152. The method according to claim 149, wherein the chemical entity can be selectively cleaved from the remainder of the building block.

153. The method according to any of the claims, wherein the chemical entity is simultaneously reacted with the reactive site and cleaved from the remainder of the building block.

154. The method according to any of the preceding claims, wherein the chemical entity is one part of an affinity pair.

155. The method according to claim 154, wherein the one part of the affinity pair is selected among biotin and dinitrophenol.

156. The method according to any of the claims 137 to 155, wherein the anticodon is protected at the 3'or/and the 5' end.

157. The method according to claim 156, wherein the protection group of the anticodon is attached to a solid support.

158. The method according any of the preceding claims, wherein the nascent building block is attached to a solid support and worked up to the final building block remaining connected to said solid support.

159. The method according to any of the claims 156 to 158, wherein the protection group is photocleable.

160. The method according to claim 159, wherein the protecting group is cleaved by exposure to UV light.

161. The method according to any claims 156 to 160, wherein a phosphate group is formed at the 5' end of the anticodon by deprotection, converting the anticodon to a substrate of a ligase.

162. The method according to any of the claims 137 to 161, wherein the reactive site is covalently attached to the template.

163. The method according to claim 162, wherein the reactive site is part of a scaffold molecule.

164. The method according to any of the claims 137 to 163, wherein the nucleic acid sequence associated with a reactive site is a building block.

165. The method according to any of the preceding claims, wherein an anticodon of a building block is ligated to a primer complementing a priming sequence of the template.

166. The method according to claim 165, wherein the primer is covalently connected to the template, thereby forming a covalent connection between the anticodon and the template.

167. The method according to any of the preceding claims, wherein the anticodon is part of an oligonucleotide further comprising a sequence complementing the framing sequence of a template or a part thereof.

168. The method according to any of the preceding claims, wherein the building blocks are incorporated stepwise.

169. The method according to any of the preceding claims, wherein the nucleic acid sequence associated with a reactive site is comprised by a nascent encoded molecule.

170. The method according to any of the claims 137 to 169 , wherein the anticodon of a building block is ligated to a preceding incorporated anticodon or a primer and the chemical entity subsequently are reacted.

171. The method according to any of the preceding claims, wherein two or more building blocks are hybridised to the template and subsequently ligated together to form a ligation product.

172. The method according to any of the preceding claims, wherein a building block is hybridised next to another building block or a primer.

173. The method according to any of the claims 137 to 171, wherein a building block is hybridised in a position spaced one or more nucleotides from another building block or primer and a spacer nucleotide is provided joining the building block with the preceding building block or the primer.

174. The method according to any of the claims 137 to 173, in which a building block being immobilized on a solid support is hybridised to a codon and subjected to a ligation reaction, followed by a detachment of the building block from the solid support.

175. The method according to any of the preceding claims, wherein the anticodon is ligated to a nucleic acid by chemical means.

176. The method according to claim 175, wherein the chemical means are selected from

a) first anticodon comprising a 3'-OH group and a second anticodon comprising a 5'phosphor-2-methylimidazole group, which are reacted to form a phosphodiester internucleoside linkage,

b) first anticodon comprising a phosphoimidazolide group at the 3'-end and a phosphoimidazolide at the 5'-end, which are reacted to form a phosphodisester internucleoside linkage,

c) first anticodon comprising a 3'-phosphorothioate group and a second anticodon comprising a 5'-iodine, which are reacted to form the internucleoside linkage 3'-O-P(=O)(OH)-S-5', and

d) first anticodon comprising a 3'-phosphorothioate group and a second anticodon comprising a 5'-tosylate, which are reacted to form the internucleoside linkage 3'-O-P(=O)(OH)-S-5'.

177. The method according to any of the preceding claims, wherein the anticodon is ligated to a nucleic acid using a ligase.

178. The method according to claim 177, wherein the ligase is selected from the group consisting of DNA ligase, RNA ligase.

179. The method according to claim 178, wherein the DNA ligase is selected among the group consisting of Taq DNA ligase, T4 DNA ligase, T7 DNA ligase, and *E. coli* DNA ligase.

180. The method according to any of the preceding claims, wherein the single stranded ligation product is obtained using denaturing conditions.

181. The method according to claim 180, wherein the denaturing conditions are obtained using a media selected from organic solvents, aprotic solvents, acidic solvents, denaturants, and alkaline solvents.

182. The method according to claim 180, wherein the denaturing conditions are obtained by heating to a temperature above the melting temperature of the duplex.

183. The method according to claims 137 to 182, wherein the single stranded ligation product is obtained by degrading the template.

184. The method according to claim 183, in which the template is degraded by any one of the methods selected from the group consisting of providing an RNA template and an RNA ligation product and treating the DNA:RNA duplex with an enzyme selected from RNAseH, RNAseA, RNAse 1, weak alkaline conditions (pH 9-10), or aqueous $Pb(Ac)_2$; providing a DNA template comprising a thiophospate in the internucleoside linker and an DNA or RNA anti-codon ligation product, and subsequent treating with aqueous iodine; and providing a DNA or RNA ligation product and a DNA template comprising an uracil nucleobase, treating with uracil-glycosylase and subsequent weak acid.

185. The method according to any of the preceding claims, wherein the single stranded ligation product is obtained by removing the template.

186. The method according to claim 185, wherein the template is removed by a process comprising cleaving any covalent link between the ligation product and the template, subjecting to denaturing conditions and separating of the template,

187. The method according to claim 186, wherein the covalent link is cleaved by a restriction endonuclease.

188. The method according to claim 185, wherein the template is separated from the ligation product by a process involving providing the template or the ligation product with a first part of an affinity pair.

189. The method according to any of the preceding claims, in which the single stranded ligation product is obtained by making the template strand double stranded.

190. The method according to claim 189, wherein the double stranded template is provided by competition hybridisation of a nucleotide similar to the ligation product, or by annealing a primer to the template and extending said primer over the extent of the template using a polymerase.

191. The method according to claims 137 to 190, wherein the reaction of the chemical entity of an incorporated building block with a reactive site is an acylation reaction.

192. The method according to claim 191, wherein the reactive site is an amine and the bond form is an amide bond.

193. The method according to any of the preceding claims, wherein one or more bond between the encoded molecule or nascent encoded molecule are cleaved.

194. The method according to claim 137, wherein steps ii) through iv) may be repeated as appropriate using a nascent complex as the template and anticodon(s) directed to a non-used codon in the building blocks to be incorporated.

195. The method according to any of the preceding claims, wherein the encoded molecule is maintained connected to the template through a single bond.

196. The method according to any of the preceding claims, wherein a plurality of templates and building blocks are processed simultaneously or sequentially forming a library of complexes.

197. The method according to claim 196, wherein pools of building blocks directed to the each codon of the plurality of templates are added sequentially.

198. The method according to claim 197, wherein the nucleotide sequences harbouring the different anticodons in each pool have an identical flanking sequence.

199. A library of different complexes, each complex comprising an encoded molecule and a template, which has encoded the chemical entities which has participated in the synthesis thereof, said library being obtainable by processing a plurality of different templates and a plurality of building blocks in accordance with any of the claims 137 to 198.

200. The method according to any of the preceding claims further comprising subjecting the library of complexes to a condition partitioning complexes displaying a predetermined property from the remainder of the library.

201. The method according to claim 200, wherein the condition for partitioning of the desired complexes includes subjecting the library of complexes to a molecular target and partitioning complexes binding to said target.

202. The method according to claim 200 or 201, wherein nucleic acid sequences comprising the codons and/or the anticodons are recovered from the partitioned complexes.

203. The method according to any of the claims 200 to 202, wherein the nucleic acid sequences of the partitioned complexes are amplified.

204. The method according to claim 203, wherein the nucleic acid sequences of the partitioned complexes are amplified using the polymerase chain reaction (PCR).

205. The method according to claim 137, wherein the amplification product is used to prepare one or more templates which may be utilized in the method of claim 137.